Food Science and Human Wellness 2 (2013) 99–104

Food Science and Human Wellness

www.elsevier.com/locate/fshw

Red onion extract (*Allium cepa* L.) supplementation improves redox balance in oxidatively stressed rats

Nidhi Jaiswal [a], Dileep Kumar [b], Syed Ibrahim Rizvi [b,*]

[a] *Centre of Food Technology, University of Allahabad, Allahabad 211002, India*
[b] *Department of Biochemistry, University of Allahabad, Allahabad 211002, India*

Received 25 December 2012; received in revised form 18 April 2013; accepted 28 May 2013

Abstract

Onions, consumed worldwide, are a good source of dietary phytochemicals with proven antioxidant properties. Catechin and quercetin are the most common and widely consumed flavonoids. The present study aimed to investigate the possible protective effect of onion extract as well as flavonoids (catechin and quercetin) on rats subjected to oxidative stress by mercuric chloride ($HgCl_2$) treatment. Experiments were conducted on rat erythrocytes, which are a good model system to study oxidative stress. Results show that the oxidative stress induced by $HgCl_2$ in Wistar rats resulted in substantially increased erythrocyte lipid peroxidation and higher activity of red cell plasma membrane redox system (PMRS) along with corresponding decrease in the intracellular reduced glutathione and antioxidant activity. Onion extract supplementation significantly ($P < 0.05$) attenuated these adverse effects of $HgCl_2$. Flavonoid supplementation resulted in a slightly higher antioxidant response compared to onion extract. We conclude that supplementation of these flavonoids results in normalization of erythrocyte PMRS activity which provides onion (rich in quercetin), a novel mechanism to exert its antioxidant effect against $HgCl_2$-induced oxidative stress in rat erythrocytes *in vivo*.

Keywords: Onion; Catechin and quercetin; Mercuric chloride; Oxidative stress; Antioxidant activity; Plasma membrane redox status (PMRS)

1. Introduction

Flavonoids are a large group of naturally occurring phenolic constituents, ubiquitously present in edible plants, vegetables and fruits. Epidemiological evidence suggests that consumption of foods and beverages rich in flavonoids correlate with lower risk of various diseases, including certain cancers, cardiovascular diseases and oxidative stress-related diseases [1]. More than 6000 varieties of flavonoids have been identified, among them quercetin (abundant in onion, apple, broccoli and berries) and catechin (abundant in tea) are the most common and widely consumed flavonoids [2]. Onion (*Allium cepa* L.), a widely consumed vegetable, is a good source of dietary phytochemical (organosulphur compounds and flavonoid especially quercetin) with proven antioxidant properties and ability to modulate the detoxification systems [3,4]. Various scientific reports have confirmed its functional properties which include free radical scavenging activities, immune stimulation, cardioprotective effects (by lowering serum cholesterol and blood pressure), anti-cancer, and anti-infectious properties [5].

Various degenerative and metabolic diseases such as diabetes, atherosclerosis, cancer and aging are known to lead toward oxidative stress [6]. In recent years, there is renewed interest toward study of plants and their isolated compounds for the prevention of diseases and diverse pathological conditions by offering protection against cellular damage and oxidative stress [7].

The present study reports the antioxidant effect of onion extract, quercetin and catechin on markers of oxidative stress in blood (plasma membrane redox system, antioxidant capacity of plasma, erythrocyte lipid peroxidation and intracellular reduced glutathione) in a rat model of experimental oxidative stress.

2. Materials and methods

2.1. Chemicals

Quercetin, (+)-catechin, 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ). 4,7-Diphenyl-1, 10-phenanthroline disulfonic acid

* Corresponding author at: Department of Biochemistry, University of Allahabad, Allahabad 211002, India. Tel.: +91 9415305910.
E-mail address: sirizvi@gmail.com (S.I. Rizvi).
Peer review under responsibility of Beijing Academy of Food Sciences.

ELSEVIER Production and hosting by Elsevier

2213-4530
http://dx.doi.org/10.1016/j.fshw.2013.05.003

disodium salt (DPI), reduced glutathione and dithiobis nitro benzoic acid (DTNB) were purchased from Sigma Aldrich, St. Louis, USA. All other analytical grade chemicals were procured from Merck, India and Himedia Labs, India.

2.2. Preparation of extract

Fresh bulbs of onions were purchased from a local market in Allahabad, India. Among the various local varieties of onion in India, the red onion (Pusa cultivar) was preferably selected because of its reported high antioxidant potential [3]. The plant was collected, its botanical identification and authentication was confirmed and the herbarium sheets were sent to the herbarium of the Department of Botany, University of Allahabad. The voucher specimen number (reference number 25840) was obtained. The bulbs were carefully dressed and frozen (4 °C). About 100 g of onion was crushed in a grinding machine with 100 mL of chilled, distilled water. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen (4 °C) until used.

2.3. Animal model and study protocol

The experiment was carried out with 48 male Wistar rats (5 ± 0.5 months and body weight 150 ± 20 g). They were housed in a temperature controlled room (25 ± 5 °C) with 12-h light–dark cycles for at least 1 week. After the stabilization period of one week, the rats were randomly divided into eight groups, containing six animals each. Onion extracts and flavonoids (catechin and quercetin) were administered by gavage for 30 days. Flavonoids under certain reaction conditions, can display prooxidant activity, thus, we selected optimal dosages for the flavonoid treatments as reported in previous studies [8]. *Group I*: Control, receiving no treatment/supplementation. *Group II*: Onion extract treated group (1 mL/100 g bw/day) for 30 days. *Group III*: Quercetin-only group, quercetin was dissolved in 0.5% DMSO and rats were treated per day *via* gavage (20 mg/kg bw) for 30 days. *Group IV*: Catechin-only group, catechin was dissolved in 0.5% DMSO and rats were treated per day *via* gavage (20 mg/kg bw) for 30 days. *Group V* (negative control): Rats were injected intra peritonealy $HgCl_2$ 5 mg/kg body weight in 0.9% NaCl [9]. *Group VI*: Onion extract plus $HgCl_2$ treated group, onion extract (1 mL/100 g bw/day) was given *via* gavage 10 days before $HgCl_2$ injection and onion extract continued for next 30 days. *Group VII*: Quercetin plus $HgCl_2$ treated group, quercetin dissolved in 0.5% DMSO (20 mg/kg bw per day) was given *via* gavage 10 days before $HgCl_2$ injection and quercetin continued up to next 30 days. *Group VIII*: Catechin plus $HgCl_2$treated group, catechin dissolved in 0.5% DMSO (20 mg/kg bw• d) was given *via* gavage 10 days before $HgCl_2$ injection and catechin continued up to 30 days.

2.4. Collection of blood, isolation of red blood cells and plasma

Rats were sacrificed under light anesthesia. Blood samples were collected by cardiac puncture into 10 unit/mL heparin. Red cells were pelleted by centrifugation at $800 \times g$ for 10 min at 4 °C. After the removal of plasma (immediately frozen at −80 °C until use for biochemical assays), buffy coat, and the upper 15% of packed RBC, the packed RBCs were washed twice with cold phosphate buffered saline (PBS) (0.9% NaCl and 10 mmoL/L Na_2HPO_4; pH 7.4) and then used for experiment. All protocols for experiments were approved by the Animal Care and Ethics Committee of University of Allahabad.

2.5. Measurement of total antioxidant activity by FRAP

The total antioxidant potential of the plasma samples was determined using a modification of the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain [10]. FRAP reagent was prepared from 300 mmoL/L acetate buffer, pH 3.6, 20 mmoL/L ferric chloride and 10 mmoL/L TPTZ made up in 40 mmoL/L hydrochloric acid. All three solutions were mixed together in the ratio 10:1:1 (v:v:v) respectively, 3 mL of FRAP reagent was mixed with 100 μL of plasma and the contents were mixed thoroughly. The absorbance was read at 593 nm at 30 s intervals for 4 min. Aqueous solution of known Fe (II) concentration in the range of 100–1000 μmoL/L was used for calibration. Regression equation of the FRAP values (μmoL Fe (II)/L) of the plasma was used for calculation.

2.6. Determination of erythrocyte malondialdehyde (MDA) content

Erythrocyte MDA was measured according to the method of Esterbauer and Cheeseman [11] with slight modification. Packed RBC (0.2 mL) were suspended in 3 mL PBS containing 0.5 mmoL/L glucose, pH 7.4. The lysate (0.2 mL) was added to 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.67% thiobarbituric acid (TBA) boiled for 20 min at 90–100 °C, cooled then the mixture was centrifuged at $1000 \times g$ for 5 min and the absorbance of supernatant was read at 532 nm. The concentration of MDA in erythrocytes was calculated using extinction coefficient ($\varepsilon = 31{,}500$) and is expressed as nmoL/mL of packed RBC.

2.7. Determination of erythrocyte GSH

Erythrocyte GSH was measured following the method of Beutler [12]. The method is based on the ability of the −SH group to reduce DTNB and form a yellow colored anionic product whose optical density is measured at 412 nm. Concentration of GSH is expressed in mg/mL packed RBCs.

2.8. Measurement of erythrocyte PMRS

The activity of the erythrocyte PMRS was estimated by the method of Avron and Shavit [13]. Briefly, PRBCs (0.2 mL) were suspended in PBS containing 5 mmoL/L glucose and 1 mmoL/L freshly prepared potassium ferricyanide to a final volume of 2.0 mL. The suspensions were incubated for 30 min at 37 °C and then centrifuged at $1800 \times g$ at 4 °C. The supernatant collected was assayed for ferrocyanide content using DPI and

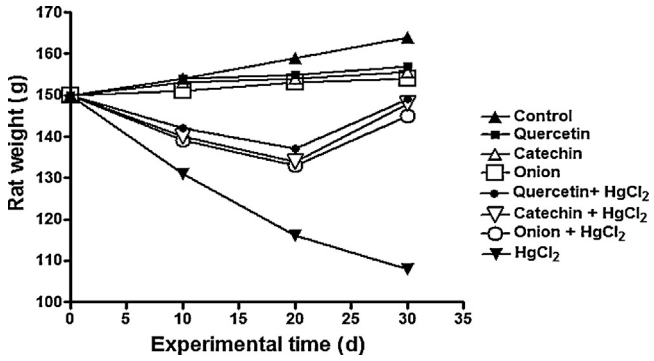

Fig. 1. Effect of mercuric chloride treatment on body weight of rats in a 30-day period.

measuring absorption at 535 nm (ϵ = 20,500/M/cm). The results are expressed in μmoL ferrocyanide/mL packed RBC/30 min.

2.9. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA.

3. Results

A significant ($P < 0.05$) reduction in body weight was observed after four weeks in mercuric chloride treated rats (Fig. 1). All the groups were studied for the plasma antioxidant capacity by FRAP assay. Treatment with either onion extract, catechin or quercetin increased the antioxidant potential of the plasma ($P < 0.05$) and ($P < 0.01$) with respect to control (Fig. 2A), however, the antioxidant potential of plasma was significantly ($P < 0.001$) reduced in rats treated with $HgCl_2$ (Fig. 2B). Treatment of $HgCl_2$ challenged rats with either onion extract, quercetin or catechin significantly ($P < 0.01$) improved the antioxidant potential of the plasma compared to $HgCl_2$ treated group (Fig. 2B).

At the end of the fourth week, the level of MDA was significantly increased in the $HgCl_2$ group compared with the control ($P < 0.001$) (Fig. 3B), and, in contrast, no statistically significant change was observed in rats treated with either onion, catechin or quercetin (Fig. 3A). The MDA level was significantly decreased in erythrocytes at the end of the fourth week in the onion + $HgCl_2$, catechin + $HgCl_2$ group and quercetin + $HgCl_2$ group compared with the $HgCl_2$ group ($P < 0.05$) and ($P < 0.001$) (Fig. 3B).

Treatment with onion extract and catechin caused slight increase (13.92% and 20.70%) in GSH level ($P < 0.05$), however, treatment with quercetin resulted in significantly higher GSH level (37.02%) as compared to control ($P < 0.001$) (Fig. 4A). $HgCl_2$ treatment significantly depleted erythrocyte GSH as compared to control ($P < 0.0001$) (Fig. 4B). Co-treatment of onion extract, catechin and quercetin with $HgCl_2$ significantly ($P < 0.001$) improved the GSH level as compared to $HgCl_2$ group (Fig. 4B).

A marked increase in erythrocyte PMRS activity was observed in $HgCl_2$ challenged rats as compared to control ($P < 0.0001$) (Fig. 5), in contrast, no significant activation of erythrocyte PMRS activity were observed when treated alone with onion, catechin and quercetin (Fig. 5A). A significant decrease in the PMRS activity was observed in $HgCl_2$ challenged rats when co-treated with onion extract ($P < 0.001$), catechin or quercetin ($P < 0.0001$) compared to $HgCl_2$ group (Fig. 5B).

4. Discussion

Oxidative stress is a deleterious condition that occurs when there is damage to cellular components, including proteins, lipids, and DNA [6]. Mercuric chloride ($HgCl_2$) is one of the most toxic forms of mercury and once absorbed into blood stream combines with proteins in the plasma or enters the red cells [14]. Previous studies have shown that mercuric chloride toxicity in rats results in the production of reactive oxygen species (ROS) which in turn generates oxidative stress [9]. In the present study, our observation of a marked reduction in body weight of rat exposed to $HgCl_2$ after 30 days is supported by previous published report [15]. Weight loss is known to be the basic aspect of mercury toxicity and has been attributed to reduced food intake.

The antioxidant capacity of plasma is the primary measure and a reliable marker to evaluate the extent of oxidative stress in induced pathological events. The present study demonstrates a decrease in antioxidant capacity in terms of FRAP value in mercuric chloride treated rats, however supplementation with flavonoids caused improvement of antioxidant potential in

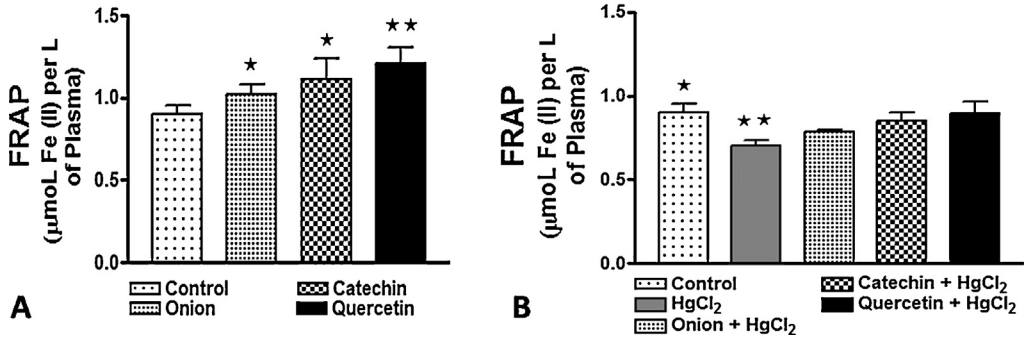

Fig. 2. Effect of onion extract, catechin and quercetin on mercuric chloride induced oxidative stress on total antioxidant capacity of plasma (measured in terms of FRAP value) *in vivo* in Wistar strain rat. FRAP value is expressed as μmoL Fe (II)/L of plasma, values represent mean ± SD.

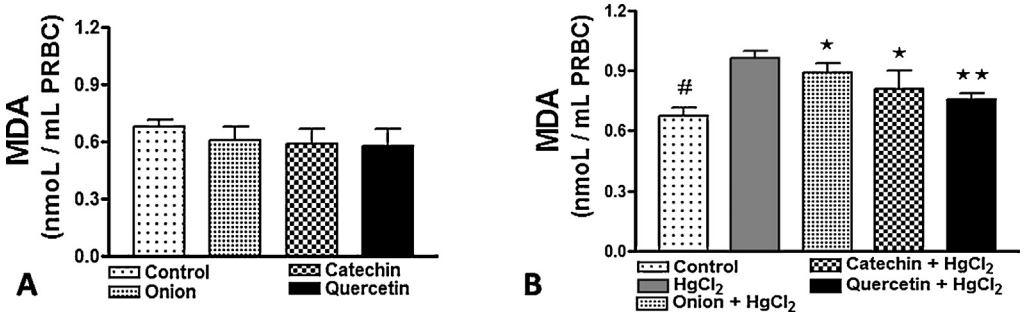

Fig. 3. Effect of onion extract, catechin and quercetin on mercuric chloride induced oxidative stress on erythrocyte malondialdehyde (MDA) content *in vivo* in Wistar strain rat. Concentration of MDA is expressed as nmoL/mL of packed RBC, values represent mean ± SD.

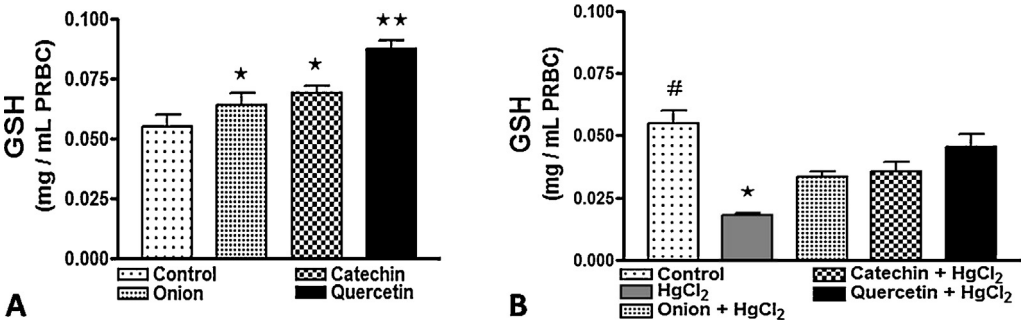

Fig. 4. Effect of onion extract, catechin and quercetin on mercuric chloride induced oxidative stress on erythrocyte reduced glutathione (GSH) content *in vivo* in Wistar strain rat. Concentration of GSH is expressed in mg/mL packed RBC, values represent mean ± SD.

$HgCl_2$ treated rats. The results are in agreement with a previous report which shows that oral administration of flavonoids (quercetin) enhanced the antioxidative ability of rat plasma, indicating that conjugated metabolites participate in the antioxidant defence [16].

Lipid peroxidation (LPO) is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of several membrane bound enzymes [17]. Under oxidative stress, the erythrocyte membrane is prone to lipid peroxidation that involves cleavage of polyunsaturated fatty acids at their double bonds, leading to the formation of MDA; an increased MDA content is an important indicator of lipid peroxidation. Increased level of MDA in erythrocyte has been reported in many disease conditions which are accompanied with oxidative stress [17]. In this study, $HgCl_2$ treatment increased lipid peroxidation by generating free radicals. This toxicity may be due to mercury-induced alterations in membrane integrity *via* the formation of reactive oxygen species by successive hydroperoxide formation and β cleavage of polyunsaturated fatty acids *in vivo* or due to perturbation of antioxidant defense mechanisms.

Reduced glutathione (GSH) is a primary intracellular antioxidant present in almost all living cells including erythrocytes, which is considered as a biomarker of redox imbalance at cellular level. The glutathione antioxidant system plays a fundamental role in cellular defence against reactive free radicals and other oxidant species. GSH depletion has been shown to intensify lipid peroxidation and predispose cells to oxidant damage [17]. A significant ($P < 0.001$) (Fig. 5B) depletion of erythrocyte GSH in $HgCl_2$ treated rats demonstrates that a condition of oxidative stress prevails in rats as a result of mercury toxicity. A single

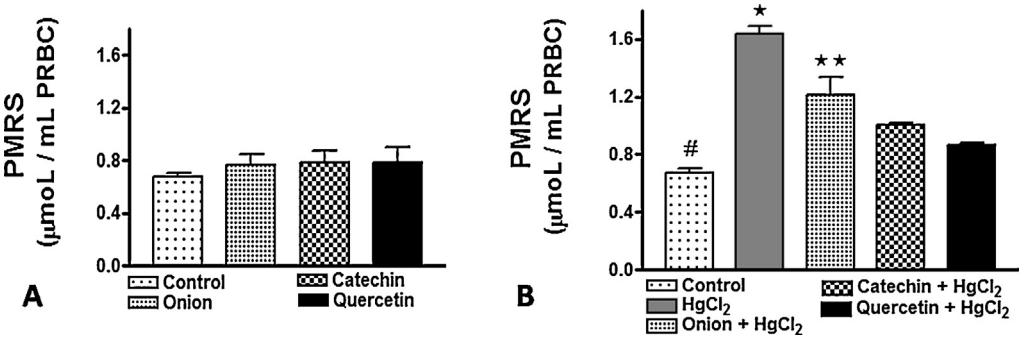

Fig. 5. Effect of onion extract, catechin and quercetin on mercuric chloride induced oxidative stress on erythrocyte plasma membrane redox system (PMRS) *in vivo* in Wistar strain rat. Activity of PMRS is expressed in µmoL ferrocyanide/mL packed RBC/30 min values represent mean ± SD.

Hg ion can bind to and cause irreversible excretion of up to two GSH molecules. The reason being that mercury has strong affinity for thiol (SH−) binding groups, especially on endogenous biomolecules [18]. Released Hg ions form complexes with GSH thereby disturbing its metabolism [14]. As a result of binding of mercury to glutathione and subsequent elimination of intracellular glutathione, levels of GSH are lowered in the cell which manifests as decrease in the antioxidant potential of the cell.

The results on the oxidative imbalance after mercury intoxication corroborates with many studies showing elevated values of MDA with decreased GSH contents in kidney and other tissues [9,15]. In a significant report blood is shown to reflect tissue oxidative stress with respect to MDA and GSH [19]. We observe a protective effect of onion extract against $HgCl_2$ induced oxidative stress evidently through marked reduction in lipid peroxidation and increased GSH content of erythrocytes and antioxidative activity of plasma. This is in agreement with previous studies which show that supplementation of onion rich diet results in increase in antioxidative ability of rat plasma [20]. The present observation may be explained due to the presence of flavonoids in red onion (especially quercetin). It is possible that the antioxidant components of the extracts might act as sacrificial antioxidants sparing the depletion of endogenous GSH during mercuric chloride-induced oxidative stress. Furthermore, dietary polyphenols have been shown to upregulate the expression of c-glutamylcysteine synthetase, the rate-limiting enzyme in the biosynthesis of GSH [21]. This may explain, in part, the enhancement of GSH in mercuric chloride-exposed rats treated with onion extracts. The high antioxidant properties of onion extract could also be attributed to the rich presence of organosulfur containing active compounds in the form of cysteine derivatives (S-methylcysteine sulfoxide) which is a rate limiting substrate in GSH biosynthesis and has also been found to be effective in preventing or ameliorating oxidative stress by scavenging free radicals [4].

The present study demonstrates that oral administration of quercetin and catechin can effectively inhibit lipid peroxidation *in vivo*, and increase antioxidant capacity in control as well as $HgCl_2$-induced oxidative stressed rats. Such protection may be due to stabilization of the erythrocyte membrane owing to the incorporation of antioxidant thereby preventing physical damage of the membrane and resulting in more efficient free radical scavenging [22]. It also involves indirect activation of transcription factors (*e.g.*, Nrf2) that regulate the expression of genes encoding for antioxidant enzymes [23]. Catechins are reported to be scavengers of superoxide radicals, peroxyl radicals and inhibitors of lipid peroxidation [8]. It chelates iron and offers superoxide scavenging and lipid peroxidation lowering properties through its structural features (catechol group in ring B and a hydroxy group in ring C). In addition, dietary flavonoids interact with phase I and phase II enzyme system, thereby modulating expression of an important enzyme glutamylcysteine synthetase, which is responsible for the synthesis of glutathione [21]. Recently, protective effect of catechin and quercetin has been highlighted on chlorpyrifos induced toxicity in rat testis tissues [24].

Eukaryotic cells including erythrocytes display a plasma membrane redox system (PMRS) that transfers electrons from intracellular substrates to extracellular electron acceptors [25]. The importance of red cell PMRS during oxidative stress has recently been highlighted [17,26]. It has been reported that PMRS is a compensatory/protective mechanism that operates to maintain the ascorbate level in plasma which is crucial for maintaining the redox balance [27]. The higher activity of red cell PMRS in $HgCl_2$ treated rats is the result of generation of oxidative stress. Our observation of the reduction of PMRS activity upon supplementation by onion extract, catechin and quercetin suggests that such treatment enhances plasma antioxidant capacity and mitigates oxidative stress in $HgCl_2$ treated rats. On the basis of PMRS results, it is apparent that quercetin is more powerful antioxidant compared to catechin and onion extract.

Quercetin and catechin have good bioavailability. Plasma total catechin concentrations are reported to range from 0.63 to 1.8 mmoL/L after ingestion of a single large dose of green tea. This plasma concentration achieved after 1.5–2.6 h returned to baseline values after 24 h [28]. Murota et al. [29] reported a plasma quercetin concentration of 1.031 μmoL/L 1.5 h after onion consumption. Significantly, it has been reported that the elimination of quercetin metabolites is very slow, with reported half-lives ranging from 17 to 28 h [30]. Thus, there is strong evidence to support that repeated intake of onion and flavonoids would lead to enteric absorption which may be sufficient to reduce plasma indices of oxidant status [31].

5. Conclusion

This study demonstrates the antioxidant effects of onion extract, catechin and quercetin against oxidative damage in rats subjected to oxidant insult by treating with $HgCl_2$. Onion extract rich in flavonoids especially quercetin could attenuate $HgCl_2$ induced oxidative stress. Supplementation of these flavonoids results in normalization of erythrocyte PMRS activity which plays a protective role in maintaining a redox state in the plasma by reducing extracellular oxidants.

Acknowledgment

Nidhi Jaiswal acknowledges the Junior Research Fellowship (JRF) from University Grants Commission (UGC), New Delhi, India.

References

[1] B.A. Graf, P.E. Milbury, J.B. Blumberg, Flavonols, flavones, flavanones, and human health: epidemiological evidence, Journal of Medicinal Food 8 (2005) 281–290.

[2] I. Erlund, Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology, Nutrition Research 24 (2004) 851–874.

[3] N. Jaiswal, S.I. Rizvi, Variation of antioxidant capacity in different layers of onion (*Allium cepa* L.) at two different stages of maturation, Current Nutrition & Food Science 8 (2012) 126–130.

[4] F.M. El-Demerdash, M.I. Yousef, N.I. Abou E.I-Naga, Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats, Food and Chemical Toxicology 43 (2005) 57–63.
[5] M. Corzo-Martinez, N. Corzo, M. Villamiel, Biological properties of onions and garlic, Trends in Food Science & Technology 18 (2007) 609–625.
[6] B. Halliwell, J.M.C. Gutteridge, Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death, in: Free Radicals in Biology and Medicine, third ed., Clarendon Press, Oxford, UK, 2007, pp. 187–267.
[7] N. Jaiswal, S.I. Rizvi, Protective effect of different layers of onion extracts (*Allium cepa* L.) on markers of oxidative stress in erythrocytes, Cell Membranes and Free Radical Research 3 (2011) 147–153.
[8] F. Demir, F.G. Uzun, D. Durak, et al., Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin, Pesticide Biochemistry and Physiology 99 (2011) 77–81.
[9] P.R. Augusti, G.M. Conterato, S. Somacal, et al., Effect of astaxanthin on kidney function impairment and oxidative stress induced by mercuric chloride in rats, Food and Chemical Toxicology 46 (2008) 212–219.
[10] I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Analytical Biochemistry 239 (1996) 70–76.
[11] H. Esterbauer, K.H. Cheeseman, Determination of aldehydic lipid peroxidation products: malondialdehyde and 4-hydroxynonenal, Methods in Enzymology 186 (1990) 407–421.
[12] E. Beutler, Red Cell Metabolism: A manual of Biochemical Methods, third ed., Grune and Stratton, New York, 1984.
[13] M. Avron, N. Shavit, A sensitive and simple method for determiination of ferrocyanide, Analytical Biochemistry 6 (1963) 549–554.
[14] L. Patrick, Mercury toxicity and antioxidants. Part 1: role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity, Alternative Medicine Review 7 (2002) 456–471.
[15] M.V. Rao, B. Chhunchha, Protective role of melatonin against the mercury induced oxidative stress in the rat thyroid, Food and Chemical Toxicology 48 (2010) 7–10.
[16] M.R. Santos, M.J. Rodríguez-Goméz, G.C. Justino, et al., Influence of the metabolic profile on the *in vivo* antioxidant activity of quercetin under a low dosage oral regimen in rats, British Journal of Pharmacology 153 (2008) 1750–1761.
[17] K.B. Pandey, S.I. Rizvi, Markers of oxidative stress in erythrocytes and plasma during aging in humans, Oxidative Medicine and Cellular Longevity 3 (2010) 2–12.
[18] B. Hultberg, A. Andersson, A. Isaksson, Interaction of metals and thiols in cell damage and glutathione distribution: potentiation of mercury toxicity by dithiothreitol, Toxicology 156 (2001) 93–100.
[19] A.S. Veskoukis, M.G. Nikolaidis, A. Kyparos, et al., Blood reflects tissue oxidative stress depending on biomarker and tissue studied, Free Radical Biology & Medicine 47 (2009) 1371–1374.
[20] K. Azuma, Y. Minami, K. Ippoushi, et al., Lowering effects of onion intake on oxidative stress biomarkers in streptozotocin-induced diabetic rats, Journal of Clinical Biochemistry and Nutrition 40 (2007) 131–140.
[21] J.O. Moskaug, H. Carlsen, M.C.W. Myhrstad, et al., Polyphenols and glutathione synthesis regulation, American Journal of Clinical Nutrition 81 (2005) 277S–283S.
[22] A. Lopez-Revuelta, J.I. Sanchez-Gallego, A. Hernandez-Hernandez, et al., Membrane cholesterol contents influence the protective effects of quercetin and rutin in erythrocytes damaged by oxidative stress, Chemico-Biological Interactions 161 (2006) 79–91.
[23] F. Arredondo, C. Echeverry, J.A. Abin-Carriquiry, et al., After cellular internalization, quercetin causes Nrf2 nuclear translocation, increases glutathione levels, and prevents neuronal death against an oxidative insult, Free Radical Biology & Medicine 49 (2010) 738–747.
[24] Y. Kalender, S. Kaya, D. Durak, et al., Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis-histoarchitecture induced by chlorpyrifos in male rats, Environmental Toxicology and Pharmacology 33 (2012) 141–148.
[25] D.H. Hyun, S.S. Emerson, D.G. Jo, et al., Calorie restriction up-regulates the plasma membrane redox system in brain cells and suppresses oxidative stress during aging, Proceedings of the National Academy of Sciences of the United States of America 103 (2006) 19908–19912.
[26] S.I. Rizvi, R. Jha, Strategies for the discovery of anti-aging compounds, Expert Opinion on Drug Discovery 8 (2011) 89–102.
[27] S.I. Rizvi, R. Jha, P.K. Maurya, Erythrocyte plasma membrane redox system in human aging, Rejuvenation Research 9 (2006) 470–474.
[28] R. Leenen, A.J. Roodenburg, L.B. Tijburg, et al., A single dose of tea with or without milk increases plasma antioxidant activity in humans, European Journal of Clinical Nutrition 54 (2000) 87–92.
[29] K. Murota, A. Hotta, H. Ido, et al., Antioxidant capacity of albumin-bound quercetin metabolites after onion consumption in humans, Journal of Medical Investigation 54 (2007) 370–374.
[30] C. Manach, G. Williamson, C. Morand, et al., Bioavailability and bioefficacy of polyphenols in humans. Review of 97 bioavailability studies, American Journal of Clinical Nutrition 81 (2005) 230S–242S.
[31] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships, Journal of Nutritional Biochemistry 13 (2002) 572–584.